



Saskatchewan
Agriculture, Food
and Rural
Revitalization

ADF

AGRICULTURE DEVELOPMENT FUND

FINAL REPORT

20020033

THE EFFECT OF INDIVIDUAL ANIMAL
TREATMENT ON THE DEVELOPMENT OF
ANTIMICROBIAL RESISTANT FECAL
BACTERIA IN FEEDLOT CATTLE

Funded by:
The Agriculture Development Fund

January, 2004
Prepared by: University of Saskatchewan



**Saskatchewan Department of Agriculture, Food and Rural
Revitalization**

Project # 20020033

**The effect of individual animal treatment on the development of
antimicrobial resistant fecal bacteria in feedlot cattle**

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Abstract

An observational trial was carried out to examine antimicrobial resistance in fecal *E. coli* isolates of cattle on arrival at the feedlot and after individual animal antimicrobial therapy in commercial feedlot conditions in Western Canada. No prophylactic antimicrobials were used in the feed during this trial. No *E. coli* isolates were found to be resistant to gentamicin or enrofloxacin in this study. On arrival, 46.2% (208/450) of isolates were susceptible to all seven antimicrobials tested. Low resistance in *E. coli* isolates was found to most antimicrobials tested. Resistance to three or more antimicrobials, out of the seven tested was found in only 3.6% (16/450) of isolates. The predominant antimicrobial resistances were to sulphamethoxazole where 38.7% (174/450) of isolates were resistant, to ampicillin where 14.7% (66/450) of isolates were resistant, and to tetracycline where 11.3% (51/450) of isolates were resistant. At the animal level, 36.6% (56/153) of isolates had all three *E. coli* isolates susceptible to all antimicrobials, and 5.9% (9/153) of animals had at least one isolate that was resistant to three or more antimicrobials out of the seven antimicrobials tested. The other predominant antimicrobial resistances were to sulphamethoxazole where 44.4% (68/153) of animals had at least one resistant isolate, to ampicillin where 20.3% (31/153) of animals had at least one resistant isolate, and to tetracycline where 17.6% (27/153) of animals had at least one resistant isolate.

In the cohort of animals examined, there were 42 treated animals and 53 not treated, for a total of 95 animals followed throughout the entire trial. Amongst these 42 treated animals there were a total of 133 Daily Defined Doses of antimicrobials used overall and only one Daily Defined Dose of antimicrobials given during the last 100 days prior to slaughter. No association was found between individual animal antimicrobial treatment and the presence of antimicrobial resistance in *E. coli* isolates preslaughter.

(Key Words: Feedlot cattle, Antimicrobial use, Antimicrobial resistance)

Executive Summary

Antimicrobial use in agriculture has come under a great deal of scrutiny because of the potential links to antimicrobial resistant infections in people. On farm food safety and quality assurance programs are increasingly becoming a requirement in order to provide quality products for consumers both within Canada and abroad. Antimicrobial use in food animals has been criticized as a potential cause of creating resistant pathogens in the food supply. However, the prudent use of antimicrobials is an important means of ensuring animal welfare and minimizing economic losses due to disease. Respiratory disease in feedlot cattle is the most common condition which requires the use of antibiotics as a method of treatment. Long acting injectable antibiotics are sometimes used in all animals at arrival to treat early cases and prevent infections from occurring. This form of treatment is known as metaphylaxis. In a previous study funded by ADF, we demonstrated that metaphylactic treatment with long acting injectable antibiotics did not have long term effects on the level of antimicrobial resistance in *E. coli* fecal bacteria in feedlot calves. This helped to show that metaphylaxis could still be considered a prudent use of antimicrobials in feedlot calves. However, there was a possibility that the individual treatments received by some animals on that project may have contributed to the levels of antibiotic resistance seen. This study endeavored to determine the level of resistance seen in calves when they arrive at the feedlot and to determine if using antibiotics for individual animal treatments had any effect on the level of resistance seen in *E. coli* fecal bacteria at the time of slaughter.

An observational trial was carried out to examine antimicrobial resistance in fecal *E. coli* isolates of cattle on arrival at the feedlot and after individual animal antimicrobial therapy in commercial feedlot conditions in Western Canada. No prophylactic antimicrobials were used in the feed during this trial. No *E. coli* isolates were found to be resistant to gentamicin or enrofloxacin in this study. On arrival, 46.2% (208/450) of isolates were susceptible to all seven antimicrobials tested. Low resistance in *E. coli* isolates was found to most antimicrobials tested. Resistance to three or more antimicrobials, out of the seven tested was found in only 3.6% (16/450) of isolates. The predominant antimicrobial resistances were to sulphamethoxazole where 38.7% (174/450) of isolates were resistant, to ampicillin where 14.7% (66/450) of isolates were resistant, and to tetracycline where 11.3% (51/450) of isolates were resistant. At the animal level, 36.6% (56/153) of isolates had all three *E. coli* isolates susceptible to all antimicrobials, and 5.9% (9/153) of animals had at least one isolate that was resistant to three or more antimicrobials out of the seven antimicrobials tested. The other predominant antimicrobial resistances were to sulphamethoxazole where 44.4% (68/153) of animals had at least one resistant isolate, to ampicillin where 20.3% (31/153) of animals had at least one resistant isolate, and to tetracycline where 17.6% (27/153) of animals had at least one resistant isolate.

In the cohort of animals examined, there were 42 treated animals and 53 not treated, for a total of 95 animals followed throughout the entire trial. Amongst these 42 treated animals there were a total of 133 Daily Defined Doses of antimicrobials used overall and only one Daily Defined Dose of antimicrobials given during the last 100 days prior to slaughter. No association was found between individual animal antimicrobial treatment and the presence of antimicrobial resistance in *E. coli* isolates preslaughter.

This study demonstrated that calves already have a proportion of antimicrobial resistant bacteria in their intestinal tract at the time they arrive in the feedlot. This "background" level of resistance is relatively low compared to surveys in other food animals such as chicken or pigs. Whether this "background" level of resistance is significant is difficult to determine. The level of resistant bacteria may be a result of antibiotic use in the cow-calf sector, or it may be a "normal" level of resistance due to exposure to environmental bacteria which would also have resistance genes present. The study has helped to create a better understanding of what this "background level" of resistance is, which will help in interpreting surveillance study findings which many government agencies are now beginning to undertake. There were also relatively low levels of multi-drug resistance in the bacterial isolates obtained at arrival. This implies that antibiotic use in the cow-calf sector has not selected for a large proportion of multi-drug resistant bacteria which could potentially be transferred to people. This provides some assurance that antibiotics are being used prudently in the cow-calf sector.

The study was unable to demonstrate any association between individual antibiotic treatments and the level of antibiotic resistance in fecal *E. coli* isolates in cattle at the time of slaughter. This has significant implications for the cattle industry as it is an initial piece of evidence that would justify the use of injectable antibiotics for the treatment of bovine respiratory disease without creating the risk of significant antimicrobial resistance that could be passed on to people. Bovine respiratory disease is the most significant infectious disease in the feedlot industry from an economic standpoint. Injectable antibiotics are an essential method for treating sick animals, improving animal welfare and avoiding catastrophic economic loss. This study

provides some initial data to show that the use of antibiotics to treat respiratory disease does not create significant changes in the resistance patterns of the fecal *E. coli* at the time of slaughter.

Technical Report

Introduction

Antimicrobial use in animals is speculated to affect human health by selecting for resistant bacteria that may then be transferred to people through the food supply and result in infections that are difficult to treat due to the acquired resistance (Smith 2002, Wegener 2003). The use of growth promotant feed antibiotics is banned in some European countries and is currently under scrutiny in Canada (Anon 1998, VDD 2002). Since the ban of growth promotants in Denmark in 1999, antimicrobial resistance in food animals in Denmark has decreased and the antimicrobial resistance in humans may show a trend downwards (WHO 2002). Health Canada has recently questioned the use of antimicrobials in agriculture (VDD 2002). Valid scientific data is required to examine the prudent use of antibiotics in agriculture. More information is also needed about the extent to which the use of antimicrobials in animal agriculture is related to antimicrobial resistance in human infections (Conly, 2002). Baseline levels of resistant bacteria in the feces of newly weaned auction market derived calves in Western Canada are not well documented yet. Previous research demonstrated that there are relatively low levels of antibiotic resistance in fecal bacterial isolates obtained from feedlot calves on arrival with some isolates with multi-drug resistance (Campbell, ADF Project 19990251). This level of resistance represents the antimicrobial resistant strains of bacteria that the calves are carrying prior to any treatment at the feedlot, and may be related to resistance patterns and antimicrobial use on their individual cow-calf operation. Understanding this background resistance is essential to understanding the changing patterns of antimicrobial resistant isolates at the feedlot, and interpreting resistance patterns preslaughter. As well, the previous study demonstrated that there might be an association between changes in antimicrobial resistance patterns in *E. coli* isolates and individual animal antibiotic treatments late in the feeding period (Campbell, ADF Project 19990251). The first objective of this study is to characterize the antimicrobial resistance patterns in fecal *E. coli* isolates of auction market derived, newly weaned cattle on arrival at the feedlot. The second objective is to investigate the relationship between individual animal antimicrobial treatment during the feeding period and the changes in patterns of antimicrobial resistance in fecal bacteria between arrival and the time immediately preslaughter.

Materials and Methods:

Trial Facilities

The University of Saskatchewan and Poundmaker feedlots served as the experimental sites for this project. Four hundred and fifty head of auction mart derived feedlot calves owned by Pound-Maker Agventures were backgrounded at the University of Saskatchewan Feedlot in 2002. Backgrounding was carried out in twelve pens at the University of Saskatchewan Research Feedlot with 8 pens of 37 animals and 4 pens of 38 animals. The cattle were moved to a large commercial feedlot (Poundmaker) with a capacity of approximately 30,000 head for the finishing period where they were housed in two pens of about 222 animals. Both feedlots are typical of those found in Western Canada with open air pens, dirt floors, a central alley, 20% porosity fencing and shared waterers between two pens. The design of the research feedlot is a small scale representative of larger regional feedlots. Both feedlots maintained individual animal records.

Animals

The University Committee on Animal Care and Supply approved this trial and the guidelines of the Canadian Council on Animal Care were followed. These cattle were enrolled in a limit feeding trial at the University of Saskatchewan feedlot and were also part of a Fusogard™ vaccine trial funded by Novartis Animal Health Canada Inc.

In the fall of 2001, 447 auction market derived steer calves arrived at the University of Saskatchewan feedlot. The steers were crossbred beef calves with an average weight of 249 kg (s = 17 kg). Routine processing was carried out at arrival which included ear tagging for individual animal identification, weighing, vaccination against infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI₃), bovine virus diarrhea (BVD), *Hemophilus somnus* and *Mannheimia hemolytica* (Starvac™ 3/Somnu-Star Ph™; Novartis Animal Health Canada Inc., Mississauga, Ontario) and vaccination against clostridial diseases (Tasvax®8; Schering Canada, Pointe-Claire, Quebec). The animals also received a topical parasiticide treatment of 1 mL/10 kg body weight (Dectomax® Pour-On; Pfizer Canada, London, Ontario) and a hormonal implant (Ralgro®, Schering Canada, Pointe-Claire, Quebec). A metaphylactic injection of antibiotics was given to each animal based on body temperature at processing. Animals with body temperatures greater than 41°C received subcutaneous injections of tilmicosin (Micotil®; Elanco Animal Health, Guelph, Ontario) at a dose of 1 mL/30 kg body weight, while the rest of the animals received subcutaneous injections of long-acting oxytetracycline (Liquamycin® LA-200; Pfizer Animal Health, Kirkland, Quebec) at a dose of 1 mL/10 kg body weight. *F. necrophorum* vaccine (Fusogard™ Novartis Animal Health Canada Inc., Mississauga, Ontario) was given subcutaneously in the neck to one half of the calves from each pen. A placebo injection of saline was given by the same method to all unvaccinated animals. All of these animal health products were used according to label instructions.

Animals were reimplanted (Component™ TE-S; Elanco Animal Health, Guelph, Ontario) and boosted for IBR and PI₃ (StarVac™ 2; Novartis Animal Health Canada Inc., Mississauga, Ontario) before moving to the finishing feedyard at 112 days on feed (DOF). A booster of *F. necrophorum* vaccine (Fusogard™; Novartis Animal Health Canada Inc., Mississauga, Ontario) was also given. A placebo injection of saline was given by the same method to all animals in the unvaccinated group.

These steers remained in their respective study groups during finishing until slaughter. Decoquate at 0.5 mg/kg BW (Deccox® 6% Premix; Alpharma, Mississauga, Ontario) was given to all trial animals. Feedlot staff checked all cattle daily for signs of disease. Any calves that appeared depressed, gaunt or distinctly different from their penmates were pulled and treated according to standard treatment protocols developed by the feedlot veterinarians. Detailed treatment records were kept on all individual animal treatments. The feedlot staff was blinded as to the allocation of the treatment groups and the specific objectives of the trial. No antibiotics were included in the feed of these cattle throughout this trial. Steers were each individually weighed at the feedlot within 24 hours of slaughter. Animals were slaughtered between 245 and 260 DOF.

Two feeding programs were used at the University feedlot during the backgrounding period to produce a gain of 1.15 to 1.25 kg/day. The diet and the feeding method were different between the two groups. One half of the animals had ad lib access to a forage based growing diet

consisting of 23.6% barley silage, 27% grass hay and 49.25% barley based concentrate/supplement on a dry matter (DM) basis. The other group was limit fed a grain based growing diet consisting of 8.5% barley silage, 92.5% barley based concentrate/supplement. Calcium, phosphorus and crude protein were adjusted so animals on either diet would have equal intakes. The feeding programs overlapped the vaccination groups in a balanced design to prevent bias. Therefore, a quarter of the animals were in each of the following treatment groups: vaccinated and limit fed, vaccinated and ad lib forage, unvaccinated and limit fed and unvaccinated and ad lib forage. The animals remained with their original diet groups throughout the second part of the feeding period but were all fed a high grain diet ad lib. The diet of the previously limit fed high grain group now consisted of 89.8% barley based concentrate/supplement and 10.2% forage (DM basis). The previously full feed high forage group was now fed a ration 90.3% barley based concentrate/supplement and 9.7% forage (DM basis).

Experimental Design

Sample size calculations indicated that 150 calf fecal samples would be sufficient to allow characterization of the antimicrobial resistance present with 80% power and a 95% confidence level. A cohort study based on antimicrobial treatments was also carried out. Animals that have been treated with antimicrobials during the feeding period were chosen for one cohort group while untreated animals were chosen for the other cohort group. Sample size for the cohort study depended on the total number of treated animals by the end of the trial, and an approximately equal sample of untreated animals would be selected as a comparison group. The necessary sample size was estimated as approximately 50 animals per group based on 80% power and a 95% confidence level.

The 447 steers were randomly allocated by blocks into twelve feedlot pens at processing. Steers were randomized within weight blocks to create approximately equal pen weights. One hundred and fifty of the 450 mixed-breed steers were chosen for fecal sampling on arrival based on a random number table. Fecal samples were collected from the rectum of each animal with an individual-use obstetrical glove. Fecal samples were collected from all calves at arrival and were stored in a -70°C freezer. The preslaughter fecal samples were placed into individual, clean foam cups with lids, labeled and transported to the lab for immediate culture.

Laboratory Analysis

The feces were thawed and cultured overnight on MacConkey's agar. Three individual strains were isolated with no further passage in vitro that might contribute to loss of plasmids coding for antimicrobial resistance. The three strains from each animal were randomly chosen to allow for strain variation within an animal, trying to get a more representative sample from the individual animal. The subcultures were cultured on blood agar. From each blood agar plate, *E. coli* were inoculated into Phosphate Buffer Sterile (PBS) to make standard solutions of 0.5 MacFarland. This solution was delivered onto Mueller-Hinton agar using the replicator technique. Each Mueller-Hinton agar plate incorporated an antimicrobial agent at a specific dilution. Each strain of *E. coli* was tested at multiple dilutions of each antimicrobial to determine the Minimal Inhibitory Concentration (MIC) of that antimicrobial. The MICs of eight antibiotics were determined using this agar dilution method. The antibiotics tested were ampicillin, tetracycline, florfenicol, sulphamethoxazole, gentamicin, enrofloxacin, trimethoprim, and

trimethoprim/sulfanilamide. The Mueller-Hinton plates were cultured at 37°C and antimicrobial sensitivities were read between 18 and 24 hours. A control strain of bacteria ATCC 25922 was included with each plate. Breakpoints and subsequent interpretations of isolates as sensitive, intermediate or resistant, for all antimicrobials used were taken from the NCCLS standards (Appendix A) (NCCLS 1999; NCCLS 2002). This panel included antimicrobials that are used in humans and livestock. All laboratory procedures were carried out according to NCCLS standards.

Missing Data

Three of the arrival samples had only 2 isolates cultured. Another three of the arrival samples had only 1 isolate cultured. An extra three samples were chosen through the same random method and laboratory analysis was performed on these. This gave a total of 450 isolates from 153 animals. Feces from seven of the treated animals were unable to be cultured at one of the time periods; therefore, 43 treated animals and 52 untreated animals were included in the cohort analysis.

Statistical Analysis

Data was entered into a commercial spreadsheet and descriptive statistics were generated (SPSS v. 11.0.1 for Windows, SPSS Inc.). The current trial was designed to evaluate outcomes at the individual animal level, which allows us to examine associations between individual animal treatments and antimicrobial resistance preslaughter. Logistic regression (SPSS v. 11.0.1 for Windows, SPSS Inc.) was used to analyze the association between individual animal antimicrobial use and antimicrobial resistance in fecal *E. coli* isolates preslaughter. The effect of clustering by pen was assessed within the logistic model, and with generalized estimating equations (SAS v.8.2 for Windows, SAS Institute, Cary, North Carolina, USA). No clustering effect was found. The assumptions of all statistical tests were met.

Results:

Arrival

The frequency of each MIC category across all *E. coli* isolates for each antimicrobial tested are presented (Figure 1, Figure 2, Figure 3, Figure 4, Figure 5, Figure 6, and Figure 7).

At the isolate level, the predominant antimicrobial resistances were to sulphamethoxazole where 38.7% (174/450) of isolates were resistant, to ampicillin where 14.7% (66/450) of isolates were resistant, and to tetracycline where 11.3% (51/450) of isolates were resistant (Table 1). No *E. coli* isolates were resistant to gentamicin, or enrofloxacin in this study. Some multi-drug resistance was found in the *E. coli* isolates (Table 2). On arrival, 46.2% (208/450) of isolates were susceptible to the seven other antimicrobials tested. Only 3.6% (16/450) of isolates were resistant to 3 or more antimicrobials.

At the animal level, the predominant antimicrobial resistances were to sulphamethoxazole where 44.4% (68/153) of animals had at least one resistant isolate, to ampicillin where 20.3% (31/153) of animals had at least one resistant isolate, and to tetracycline where 17.6% (27/153) of animals had at least one resistant isolate (Table 3). Multiresistance is described at the animal level as well (Table 4). Of all animals, 36.6% (56/153) had all three *E. coli* isolates susceptible to all antimicrobials, and 5.9% (9/153) of animals had at least one isolate resistant to three or more

antimicrobials out of the seven antimicrobials tested. The antibiograms or resistance phenotypes are presented (Table 5).

Individual Animal Treatments

The measurement, DDD, was used to describe the number of antimicrobial treatments received per pen at the approved dose of the antimicrobial. This measurement accounted for the dosage and duration of action of the antibiotic (Table 6). The concept of DDD is based on that used in the human literature (Austin 1997, Austin 1999; Baquero 1996).

In the two cohorts of animals examined, there were 42 animals that received individual antibiotic treatments and 53 animals sampled as a comparison group which did not receive any antimicrobial treatments. Among the 42 treated animals there were a total of 133 Daily Defined Doses of antimicrobials used overall and only one treatment in the last 100 days immediately prior to slaughter. At arrival, there were 119 *E. coli* isolates resistant to ampicillin and 31 resistant to tetracycline and 104 resistant to sulphamethoxazole. At the preslaughter time period, there were 249 *E. coli* isolates resistant to ampicillin and 214 resistant to tetracycline and only 50 resistant to sulphamethoxazole; this is further broken down by treatment group (Table 7).

There were three *E. coli* isolates cultured from each animal at both time periods (arrival and preslaughter). Each animal was described by the number of resistance isolates to each antimicrobial at each time period, and the change in number of isolates classified as resistant between arrival and slaughter is graphed (Figure 8, Figure 9, Figure 10, Figure 11, Figure 12, Figure 13).

The binary outcome indicating a change in resistance patterns when an animal had at least one resistant isolate to a specific antimicrobial at Time 2 after not having at least one resistant isolate to a specific antimicrobial at Time 1 was examined for each of the five antimicrobials where antimicrobial resistance was found in this study: ampicillin, tetracycline, sulphamethoxazole, trimethoprim/sulfa, and trimethoprim (Table 8). Total DDD, tilmicosin DDD, excenel DDD and treatment during the last 100 days on feed were investigated as risk factors in a logistic regression model. No associations were found. Another binary outcome indicating a change in resistance patterns when an animal had at least one isolate resistant to three or more antimicrobials at Time 2 after not having at least one isolate resistant to three or more antimicrobials at Time 1, was also examined. The same risk factors were examined and no associations were found between antimicrobial use and the antimicrobial resistance. Adjustment for potential clustering by pen did not affect the results.

Discussion

E. coli has been chosen as the indicator organism in this trial because it is a commensal bacteria in cattle that is easy to culture. It is also one of the major carcass contaminants at slaughter, and could potentially transfer resistance to other zoonotic or commensal organisms that might colonize people. It was judged important to ensure this last fecal sample was collected during the last 24 hours prior to shipping for slaughter. This timing is potentially important when looking at the risk of antimicrobial resistance transferring to people via the food chain.

Arrival

Calves arrive at the feedlot with relatively low proportions of resistant bacterial isolates. There were 14.7% (66/450) isolates resistant to ampicillin, 11.3% (51/450) isolates resistant to tetracycline, and 38.7% (174/450) isolates resistant to sulphamethoxazole. At the animal level, this was 20.3% (30/153) of calves with resistance to ampicillin, 17.6% (27/153) of calves with resistance to tetracycline and 44.4% (68/153) of calves with resistance to sulphamethoxazole. There was no resistance found to enrofloxacin and gentamicin, both of which are not approved for use in cattle in Canada. These proportions of resistant isolates are higher than those found in our previous study, where the proportions of isolates resistant to each of these three antimicrobials were less than 10% (Campbell, ADF Project 19990251). This difference may be due to the less uniform group of newly weaned auction market derived calves fed in the current study compared to the previous one. The proportion of tetracycline resistant *E. coli* isolates is similar to that found in newly weaned calves on pasture in an American study where 13 to 17% of fecal samples contained *E. coli* resistant to tetracycline in at least one of the five isolates per fecal sample (Huston 2003). The current study allows us to broaden our knowledge on the range in the proportion of resistance present on arrival using three isolates per animal to characterize this resistance.

Individual Animal Treatments

Overall this group of commercial cattle did not require a large number of antimicrobial treatments, limiting the power of the study. No associations were found in this study with individual animal antimicrobial use and antimicrobial resistance, even for the more uncommon antimicrobials we studied to which an isolates could be resistant. This is somewhat different than findings from other studies where associations were found between antimicrobial resistance in fecal *E. coli* isolates in pigs and cattle and individual antimicrobial use when used in addition to feed antimicrobials (Dunlop 1998, O'Connor 2002). In the pig study, individual animal use of gentamicin was associated with the farm-level prevalence of gentamicin resistant in fecal *E. coli* isolates (Dunlop 1998). In the cattle study, the use of injectable oxytetracycline in individual animals receiving chlortetracycline in the feed was associated with increased prevalence of resistance to chloramphenicol and sulfisoxazole in fecal *E. coli* isolates (O'Connor 2002). In both cases, the associations were found with antimicrobials not used for mass medication of animals in the feed. It was suggested that an association of antimicrobial resistance with feed medication might obscure the relationship between individual animal antimicrobial treatment and antimicrobial resistance if this relationship did indeed exist (Dunlop 1998, O'Connor 2002). The current study had no antimicrobials used in the feed during the entire feeding period but resistance profiles were performed for only a limited number of antimicrobials used in humans and cattle.

In conclusion, the study demonstrated the relatively low levels of resistant bacteria that are prevalent in *E. coli* isolates cultured from beef calves at weaning. Calves which received injectable antimicrobials during the feeding period as a result of becoming sick did not have higher levels of resistant bacteria when compared to calves that were not treated. It would appear that using injectable antibiotics in a prudent fashion during the feeding period did not increase the proportion of resistant fecal *E. coli* isolates at the time of slaughter.

Project Activities

This study has been recently presented at the International Society of Veterinary Epidemiology and Economics in Vina del Mar, Chile. Other presentations will be planned at veterinary meetings such as the World Buiatrics Meeting and the American Association of Bovine Practitioners. A paper is currently being prepared for publication in the scientific literature. In each case, the funding by Saskatchewan Agriculture and Food was gratefully acknowledged.

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Appendix A: Concentration range and breakpoints of antimicrobials tested (in µg/ml.)

Antimicrobial	Breakpoint for Resistant	Concentration Range Measured
Ampicillin	≥32	<4, 4, 8, 16, 32, >32
Enrofloxacin	≥2	<0.5, 0.5, 1, 2, 4, >4
Florfenicol	≥8	<8, 8, 16, 32, 64, >64
Gentamicin	≥16	<4, 4, 8, 16, 32, >32
Sulphamethoxazole	≥512	<125, 125, 256, 512, >512
Tetracycline	≥16	<2, 2, 4, 8, 16, 32, >32
Trimethoprim	≥16	<4, 4, 8, 16, 32, >32
Trimethoprim/ Sulfanilamide	≥4/76	<1/19, 1/19, 2/38, 4/76, 8/152, >8/152

Figure 1: The distribution of MICs for ampicillin in all *E. coli* isolates on arrival at the feedlot.

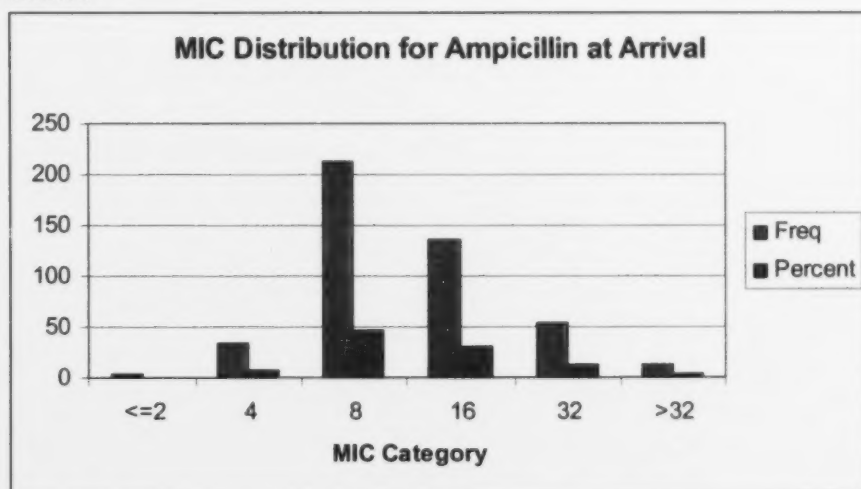


Figure 2: The distribution of MICs for tetracycline in all *E. coli* isolates on arrival at the feedlot.

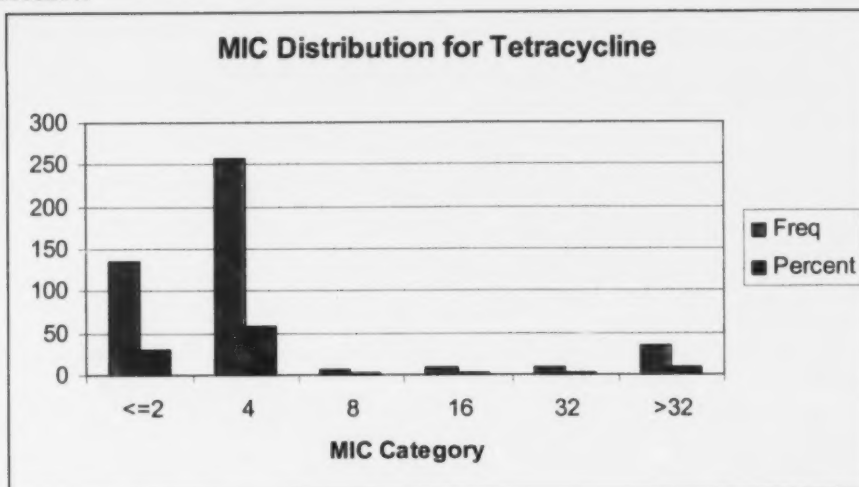


Figure 3: The distribution of MICs for sulphamethoxazole in all *E. coli* isolates on arrival at the feedlot.

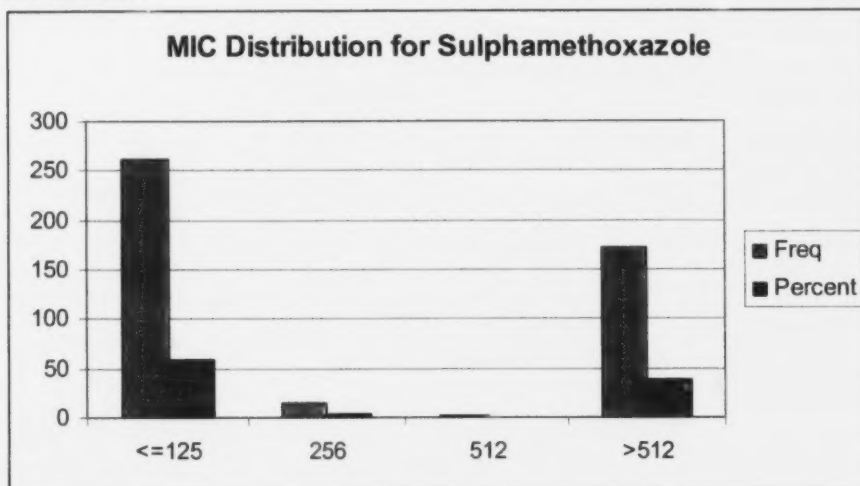


Figure 4: The distribution of MICs for gentamicin in all *E. coli* isolates on arrival at the feedlot.

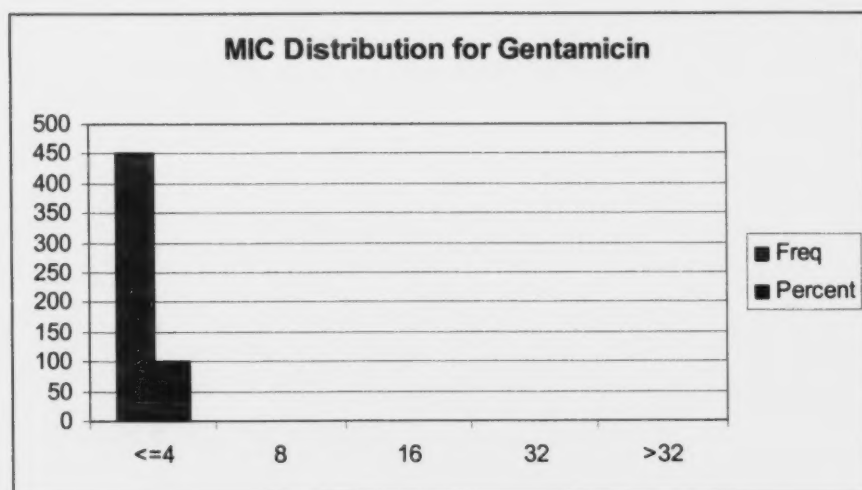


Figure 5: The distribution of MICs for enrofloxacin in all *E. coli* isolates on arrival at the feedlot.

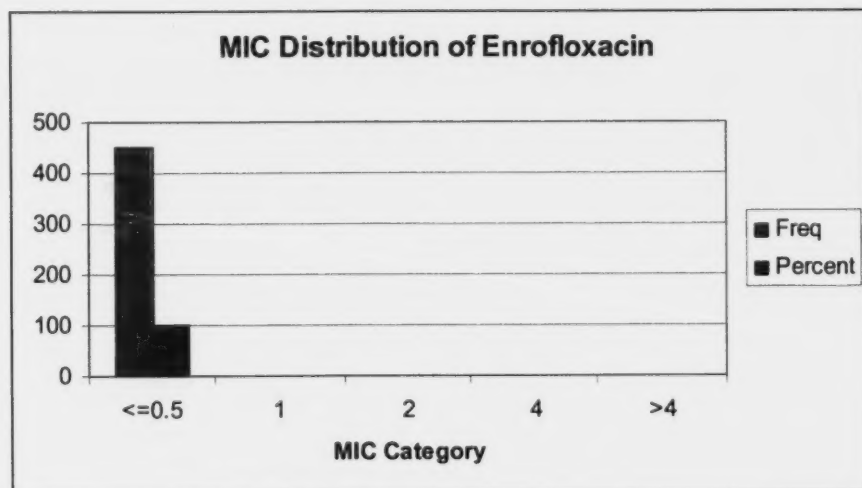


Figure 6: The distribution of MICs for trimethoprim/sulfanilamide in all *E. coli* isolates on arrival at the feedlot.

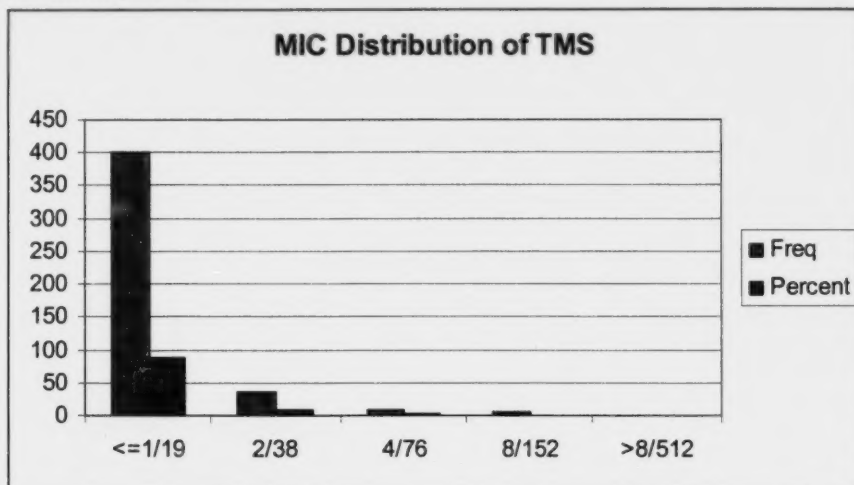


Figure 7: The distribution of MICs for trimethoprim in all *E. coli* isolates on arrival at the feedlot.

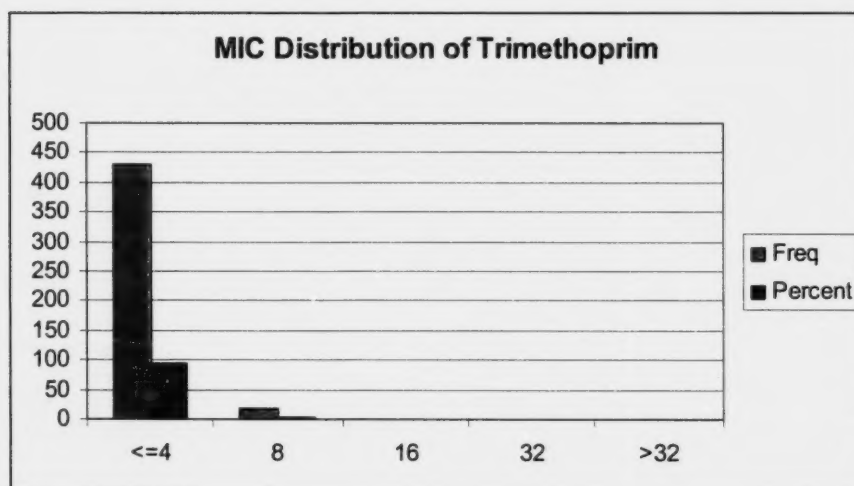


Table 1: Antimicrobial resistance of *E. coli* isolates

	Number of isolates resistant out of 450 (%)
Ampicillin	66 (14.7)
Tetracycline	51 (11.3)
Sulphamethoxazole	174 (38.7)
Gentamicin	0
Enrofloxacin	0
Trimethoprim	1 (0.2)
Trimethoprim/Sulfanilamide	13 (2.9)

Table 2: Number of antimicrobials each *E. coli* isolate is resistance to

Number of antimicrobials	0	1	2	3	4
Frequency out of 450 (%)	208 (46.2)	195 (43.3)	31 (6.9)	16 (3.6)	0

Table 3: Antimicrobial resistance of animals. An animal is considered positive if one or more isolate is resistant to the antimicrobial.

	Number of animals resistant out of 153 (%)
Ampicillin	31 (20.3)
Tetracycline	27 (17.6)
Sulphamethoxazole	68 (44.4)
Gentamicin	0
Enrofloxacin	0
Trimethoprim	1 (0.7)
Trimethoprim/Sulfanilamide	7 (4.6)

Table 4: Number of antimicrobials each animal is resistance to based on one isolate per animal with resistance to the most number of isolates

Number of antimicrobials	0	1	2	3+	4
Frequency (%)	56 (36.6)	71 (46.4)	17 (11.1)	9 (5.9)	0

Table 5: Phenotype patterns of *E. coli* isolates.

Phenotype	# Isolates (%)
None	208
S	130
A	53
Tet	12
AS	1
ATet	3
STms	7
TetS	20
ATetS	9
TetSTr	1
TetSTms	6
Total	450

Legend:

A-Ampicillin

Tet-Tetracycline

S-Sulphamethoxazole

Tms- Trimethoprim/Sulfanilamide

Tr-Trimethoprim

Table 6: Antimicrobials used in this study and Defined Daily Dose (DDD) equivalent

Antimicrobials used in study (Concentration)	Dose equivalent	DDD equivalent (days)
Ceftiofur 50 mg/mL Intramuscular injection (Excenel® Sterile Powder, Pharmacia Animal Health, Orangeville, ON, Canada)	1mg/kg BW ^a	1
Oxytetracycline 200 mg/mL Intramuscular injection (Liquamycin® LA-200, Pfizer Canada Inc., Kirkland, QC, Canada)	20 mg/kg BW	3
Tilmicosin 300 mg/mL Subcutaneous injection (Micotil, Provel, Guelph, ON, Canada)	10 mg/kg BW	3

^aBody Weight (BW)**Table 7: Number of isolates resistant to specific antimicrobials at arrival (time 1) and preslaughter (time 2).**

Time	Tx	Amp*	Tet	Sulpha	TMS	Tri	MR3plus	N
1	none	122	31	107	10	1	11	279
2	yes	130	101	15	0	0	15	144
	no	119	111	35	4	5	33	139
Total		368	243	157	14	6	49	

*Amp=ampicillin, Tet=tetracycline, Sulpha=sulphamethoxazole, TMS=trimethoprim/sulphamethoxazole,
Tri=trimethoprim, MR3plus=resistant to 3 or more antimicrobials

Table 8: Number of animals that had at least one resistant isolate present preslaughter after not having any resistant at arrival.

Treatment	Ampicillin	Tetracycline	Sulphamethoxazole	Trimethoprim/Sulfa	Trimethoprim	N
Yes	29	35	5	0	0	43
No	27	43	8	2	3	52
Total	56	87	13	2	3	95

Figure 8: The change in the number of tetracycline resistant isolates within animals between arrival and preslaughter sampling

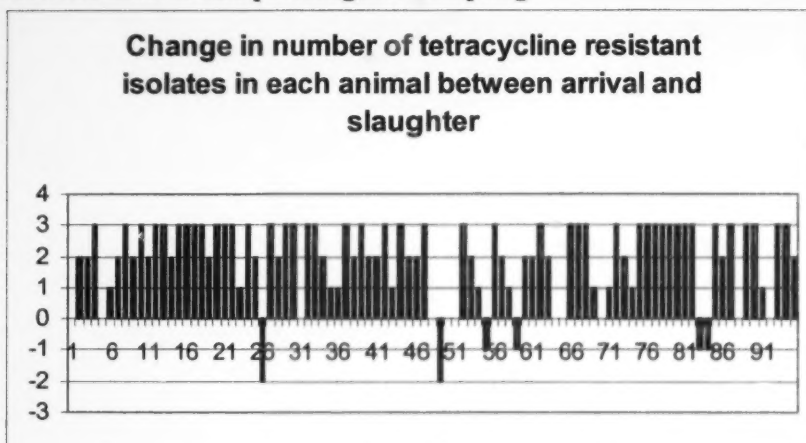


Figure 9: The change in the number of ampicillin resistant isolates within animals between arrival and preslaughter sampling

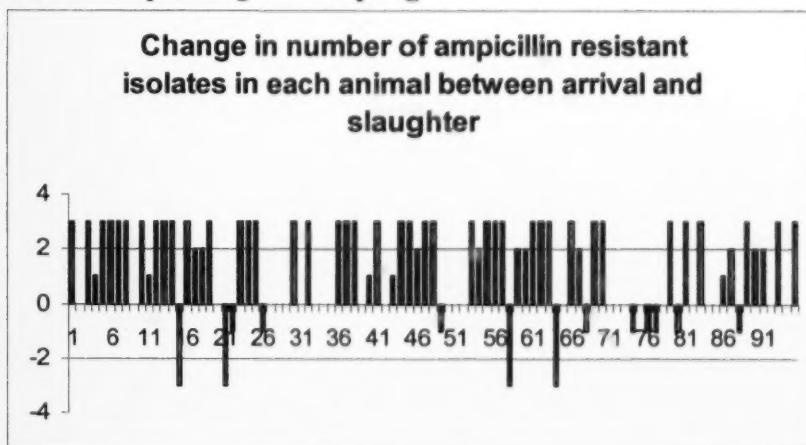


Figure 10: The change in the number of sulphamethoxazole resistant isolates within animals between arrival and preslaughter sampling

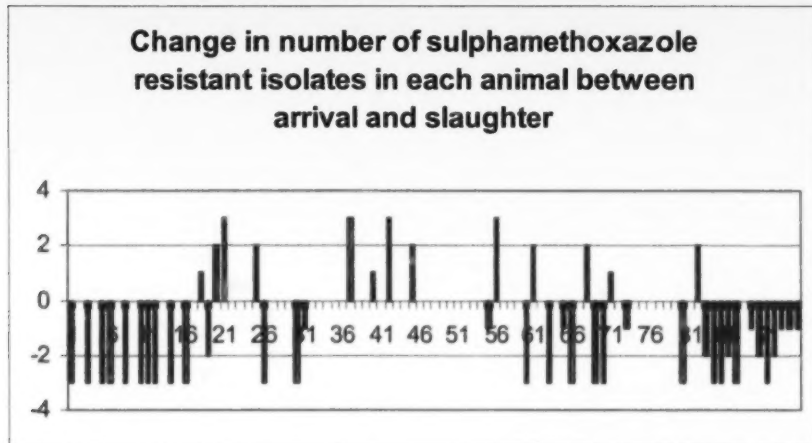


Figure 11: The change in the number of Trimethoprim-Sulfa resistant isolates within animals between arrival and preslaughter sampling

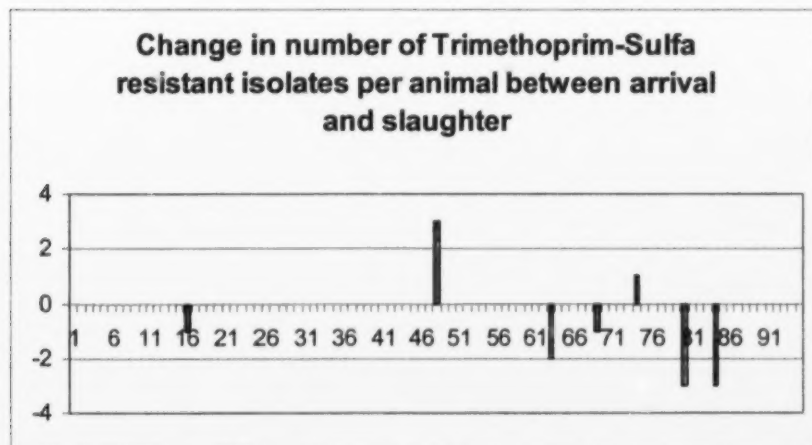


Figure 10: The change in the number of sulphamethoxazole resistant isolates between arrival and preslaughter sampling

Change in number of sulphamethoxazole resistant isolates in each animal between arrival and slaughter

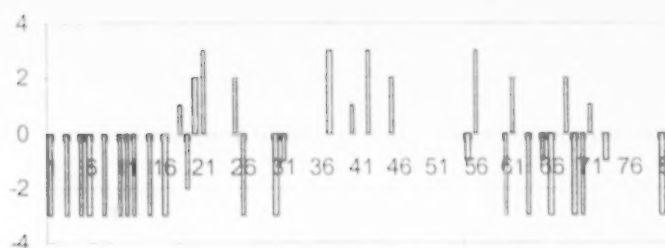


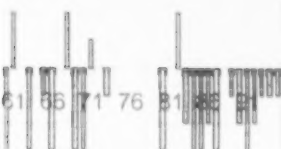
Figure 11: The change in the number of Trimethoprim-Sulfa resistant isolates between arrival and preslaughter sampling

Change in number of Trimethoprim-Sulfa resistant isolates per animal between arrival and slaughter



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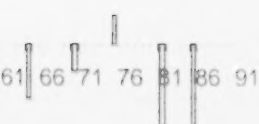


Figure 12: The change in the number of Trimethoprim resistant isolates within animals between arrival and preslaughter sampling

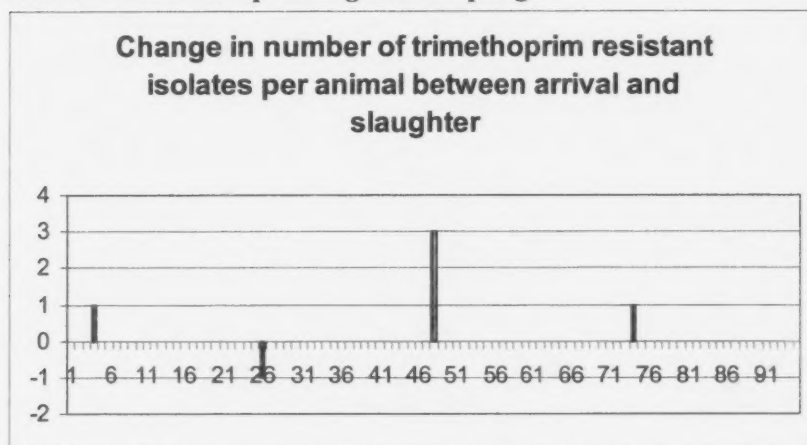


Figure 13: The change in the number of multi-drug resistant isolates within animals between arrival and preslaughter sampling

